a well marked constriction in the macrospore is due simply to a slightly diminished development of the outer layers of the spore-wall in the upper part of the spore, and a trace of this can always be found in the older spores. At any rate the two species must be regarded as very closely related.

Explanation of Plate CXLVI.

The magnification is indicated before each figure.

Fig. 1. The base of a very young fertile leaf of *Pilularia Americana*, showing the beginning of the sporocarp, m. its apical cell.

Fig. 2. Longitudinal section of an older sporocarp—F. F. two of t e young lobes.

Fig. 3. A similar section of a somewhat older sporocarp.

Fig. 4. A still older sporocarp, in which the cavities are well developed.

Fig. 5. Longitudinal section of on older sporocarp, including the peduncle. S. S. sori; H. hairs; fb. fibro-vascular bundle of the peduncle.

Fig. 6. Longitudinal section of about the same age as in Fig. 5, but at right angles to the peduncle.

Fig. 7. Longitudinal section of the young sorus, a. b. c. young sporangia.

Fig. 8. Transverse section of an older sporocarp, showing the four cavities.

Fig. 9. A single cavity from a somewhat younger one.

Figs. 10, 11. Young sporangia, t. the tapetum.

Fig. 12. Part of the wall of a nearly full grown sporocarp, a. b. c. the outer thick-walled cells.

Free Nitrogen Assimilation by Plants.

BY H. W. CONN.

The study of bacteriology has introduced to us an entirely new realm of knowledge. Twenty-five years ago the scientific world had little conception of the great change that was to be made in our knowledge of the processes of nature by the development of the study of micro-organisms. That bacteria were the cause of certain diseases was even then strongly believed by many and had been definitely proved in a few cases. But that micro-organisms, in general, lay at the foundation of many of the most important physiological processes of nature was not even dreamed of. The difficulty of research in this line made it possible for only a very

few to accomplish anything. About ten years ago Robert Koch invented a method of obtaining pure cultures which has placed this line of investigation within the reach of all. The simplification of the methods of obtaining pure cultures has produced an immense stimulus in this branch of study and has turned hundreds of observers towards investigation in this direction. The study of the last ten years has been modifying our ideas with marvelous rapidity. That bacteria produce disease is demonstrated beyond question, but we are beginning to realize that this is only a small part of the purposes they fulfill. We are learning that it is to the action of these organisms that many of the normal processes in nature are due, and that it is to their agency that the growth of plant life of the higher orders is largely indebted. One of the most recent and most surprising discoveries has been the relation of bacteria to the process of nitrogen assimilation.

It is well known to all botanists that nitrogen is an absolutely necessary food for plants and animals. It is known further that the animal kingdom obtains all of its nitrogen from plants, and that plants obtain their nitrogen from the soil in the form of certain salts, the chief of which are nitrates. Now, it is an undoubted fact, that the nitrogen factor of the soil upon which plants can depend, is little by little becoming exhausted through various agencies. In the first place, all of the nitrogenous parts of plants which are used by man as food, find their way eventually into the sewage, thence to the river, and finally to the ocean; and once reaching the ocean, the nitrogen is lost, so far as the terrestrial vegetation is concerned. Moreover, it has been shown that the various processes of putrefaction are slowly turning the complex nitrogen compounds into simpler ones, and eventually eliminating the nitrogen into the air in a free form. Inasmuch as all organic compounds, animal and vegetable alike, are subject to these putrefactive agencies, it is evident that a large amount of the nitrogen factor of the soil must, in this way, pass out into the air as free nitrogen. The extent of this loss is unknown, but it is doubtless considerable. Plainly, unless there is some way of getting the nitrogen back from the air to the soil again, the soil is doomed to exhaustion. But it has seemed to be the result of many experiments that plants in their ordinary vegetation are capable of ob-

taining nitrogen from the soil alone, and are unable to use the free nitrogen of the atmosphere. Putting all of these facts together, it has seemed to science that the nitrogen store upon which plants can draw is being used up and must be sometime exhausted, thus putting an end to vegetation.

Practically our agriculturists have for some time experienced the difficulties arising from this source. Many soils under long cultivation have become largely exhausted of their nitrogen supply, and the farmer appreciates more and more the necessity of nitrogen fertilizers. These are now brought from long distances. The nitrate beds of Chili and the guano beds of the South Pacific are the chief stores from which this valued food is obtained. But even the nitrogen beds have their limits, and as the need for nitrogen on our cultivated soils becomes greater through exhaustion, the price of nitrogen must become greater also. One writer has said that the explosion of powder in a gun does more injury than the bullet. The latter only kills a man, the former aids in in using up the nitrogen store which cannot be replaced and is a lasting injury to mankind. It is very plain from all of this that there is a great need for some means of obtaining nitrogen for our soils besides the store of nitrates in our nitrate beds. Curiously enough, the bacteriologists are to-day pointing out the method by which this problem can be solved.

As mentioned above, it was the conclusion of many experiments that the higher plants cannot make use of free nitrogen from the air. Up to 1880 all experiments seemed to point to the same direction. At about that period, however, experiments with certain of the legumes began to show an increase in the nitrogen in plants beyond that which was fed to them in their food. The experiments were at first rather indefinite and strongly denied, but as the decade from 1880 to 1890 passed they became more numerous and conclusive until, finally, it was a definitely established fact that many of the legumes can in some way obtain nitrogen from some source besides the soil. In 1888 Hellriegel and Wilfarth, in a series of careful experiments upon the subject, studied the relation of this nitrogen assimilation to the production of tubercles upon the roots of plants. Pea plants and other legumes have been for a long time known to develop small nodules

on their roots, and experiments of Hellriegel and others showed a parallel between the development of these root tubercles and the power of obtaining nitrogen in large quantities. It was Hellriegel, also, who first demonstrated that these root tubercles were abnormal products on the roots of the pea, produced there by certain organisms in the soil. Hellriegel found, for instance, that peas growing in sterilized soil produced no tubercles and fixed no nitrogen, while peas growing in a similar sterilized soil but watered with water in which ordinary soil had been standing for awhile (soil infusion), did develop tubercles and fixed nitrogen. The same soil infusion when sterilized was not able to cause the production of tubercles. From all of this it was evident that the tubercles were produced upon the roots of the peas through the agency of some of the organisms in the soil.

A microscopic study of these root tubercles soon attested the same conclusion. It was found that the tuberacles were filled with small organisms related to bacteria, and that the development of the tubercles was parallel to the developement of these organisms. The organisms are somewhat different from normal bacteria and have been called bacteroids.

According to the investigations of Prazmowski in 1890, the development and growth of the tubercles are as follows: *Bacterium radiciola* lives normally in the earth and collects in numbers on the outside of the roots of various legumes. Some of the organisms succeed in forcing their way into the tissues of the young roots, though they are not able to pierce the older roots. For a while they may remain in the root as free bacteria, but the plant plasma seems to exert an injurious influence upon them, for very soon a thin membrane is formed around the bacteria masses, inclosing them like a pouch. Prazmowski thinks that this membrane is a product of the bacteria themselves, formed for the purpose of protecting them from the injurious action of the plant tissue. The bacteria, which do not succeed in getting into one of these pouches, soon cease to grow and degenerate into irregular forms called bacteroids. The bulk of the bacteria, however, become inclosed in the membrane, after which they continue their growth with much vigor. The pouches begin to grow in thread-like masses, and these make their way among the cells of the root.

The thread branches more or less as it lengthens, and its various filiments grow through and between the cells, soon permeating the root with a fine, branching filament, which looks much like the mycelium of a mould. This bacteria pouch has been regarded as the hypha of some low fungus, but instead of being a mycelium growth of a mould the thread is nothing more than a large branching colony of bacteria enclosed in a thin membrane.

The growth of this colony of bacteria among the cells of the root stimulates these cells to an unusual growth. They multiply more rapidly than usual, and thus soon produce a swelling on the root which is the beginning of the tubercle. While this rapid multiplication of root cells is going on, the bacteria pouch continues to grow and swells out into rounded vesicles within the cells which lie at the center of the forming tubercle, until most of them become filled with these expanded portions of the bacteria thread. Meantime the root cells of the plant have been rapidly growing, and form around the cells containing the bacteria, several layers of smaller cells, which develop into a hard, corky covering, forming a coat around the tubercle. This seems to be impervious to the bacteria thread, and confines the bacteria within its limits.

The bacteria colony now undergoes a change. Although Prazmowski has not been able to follow the details of the process, it is thought that the vesicles in the central cells swell until the membrane covering the bacteria is so thin that it bursts, and the bacteria are themselves extruded into the plasma of the root cells. At all events the vesicles disappear and there appears in their place what is called the bacteroid tissue. His interpretation is that the vesicles burst, and the bacteria coming into the cell plasma are immediately checked in their growth by the injurious influence of this plasma and begin to undergo involution changes. Instead of multiplying in the normal manner, they assume various abnormal forms which have no further power of growth. They become, in short, the bacteroids which have been found by many observers, filling the central cells of the tubercle. The bacteria retain their power of growth only so long as they remain in the protecting covering of the membrane.

The tubercle by this time is pretty well formed. The outer cells have undergone quite an extended growth and differentiation,

so that the tubercle is really a structure of a rather high grade of plant tissue. The tubercle itself is thus really a growth of the root cells of the plant and not a growth of bacteria. In the center of this mass of plant tissue are a large number of cells which are completely filled with the so-called bacteroids. These bacteroids give to the tubercle at this stage a flesh-red color. Some of these central cells are so completely filled with them that nothing else can be seen, while others may still show the cell nucleus. In others spaces begin to appear in the body of the cell. The appearance of the spaces marks a new stage in the history of the tubercle and indicates that the bacteroids are beginning to be absorbed by the plant. The cell plasma soon assumes a network structure, and from this time the bacteroids entirely cease their activities and begin to disappear rapidly. After a little they are completely absorbed by the substance of the plant, and the tubercles are left as empty pouches. The tubercles have now changed their appearance again and assume a somewhat grayish green color.

This practically ends the history of the tubercle. In most cases some of the bacteria seem to remain within their original membrane and, therefore, are still capable of growing. These may or may not set up a secondary growth, but it amounts to little, for by this time time the plant has usually blossomed, ripened its seeds, and the root is beginning to die. The tubercle is immediately attacked by the putrefactive bacteria in the soil and becomes decomposed.

Since the work of Prazmowski other observers have studied the same problem. Pure cultures of the root organism have been obtained and used by artificial inoculation. Water cultures have been made where the process can be better studied. The general result has confirmed the idea above outlined, and proved conclusively that the tubercles are the result of the action of micro-organisms in the soil.

There has been more or less dispute in regard to the actual nature of these organisms. For a time they were thought to be parts of the roots of the legumes, but this idea was soon abandoned, and they were regarded as the hyphæ of moulds. Later their relation to bacteria was rendered extremely probable, and

this is the generally accepted view to-day. The organisms do not act like ordinary bacteria, since they grow in a different way and have a somewhat different form. It is a matter of comparatively little importance, however, whether the organisms are regarded as true bacteria or simply as related organisms. The significant fact is that they are colorless microscopic organisms, living in the soil, belonging to the low fungi and having the remarkable functions above pointed out.

The work of the last two years has shown further that there are a number of different species of these root organisms and that different species of legumes are associated with different species of these bacteroids. The organism which produces the root tubercles of the lupine will not produce the root tubercle of the pea, and, although the subject has not been as yet very thoroughly cleared up or studied very widely, it seems that nearly all of the different species of legumes are associated with different forms of organisms in the soil. It has followed from this that special soils are especially adapted for the growth of certain species of legumes. A soil for instance in which the lupine has been growing is much better adapted for the production of tubercles on lupine roots than it is for the production of tubercles on the roots of the pea, simply because the soil is already filled with the organisms which can grow in the lupine and not yet provided with that growing in the pea. It requires thus a culture of a year or two to develop in the soil a sufficient quantity of the appropriate species of bacteria to render the growth of any species of legume especially advantageous. The special work of bacteriologists at the present time is turned largely in the direction of determining the facts in regard to this matter of bacteria species associated with the different species of legumes.

It is plain from the above that the production of root tubercles is not a normal feature of the life of the pea plant, and that the bacteria have some peculiar relation to the higher organism. It is, however, hardly proper to regard their relation as that of a parasite and its host. It is true that the bacteria grow in the root of the legume and doubtless obtain sustenance therefrom, but the higher organism does not suffer from the parasitism and the relation of the two organisms is rather that which is known as symbiosis, i. e., a

relation in which each organism gains advantage. So far as the bacteria are concerned, they doubtless gain a place for developing a breeding pouch, and perhaps gain some sustenance from the root of the pea. So far as the pea plant is concerned, the presence of the organism makes possible the assimilation of free nitrogen. In all of the experiments which have been carried on it has been found that the production of the tubercles is necessary to enable the legumes to assimilate nitrogen. Where the legumes develop without tabercles on account of the lack of bacteria in the soil, no nitrogen was assimilated, and where the tubercles were very abundant, much nitrogen was taken from the air. It is plain then that the pea plant obtains a considerable advantage from its association with the lower organism.

As to the method by means of which this association of organisms extracts the free nitrogen from the air, we are as yet in the dark. That it is free nitrogen that is assimilated by the plant and not combined nitrogen, has been demonstrated by the experiments of the last two years, but where the nitrogen is first fixed is as yet a question. It has been suggested that the bacteria themselves take the nitrogen out of the air and store it up in these tubercles; it has been suggested that the bacteria stimulate the legumes in such a way as to enable the legume to seize the free nitrogen from the air and store it in the roots; and it has been suggested that the assimilation of the nitrogen is a matter of the combined action of the bacteria and the legume life together. Which of these possibilities is the proper one science has not yet indicated, but it has been satisfactorily proved that through the combined life of the bacteria in the root of the pea plant nitrogen is taken from the air and stored up in the roots of the pea plant in the form of nitrogen compounds of high complexity.

The work on this subject of nitrogen assimilation at the outset seemed to indicate that the family of legumes alone possess the power of absorbing nitrogen from the air. Undoubtedly, this family possesses this power to a greater extent than any other family of plants, but it is still a question whether the same power is not developed in certain other plants. Upon this matter experimenters are not in agreement at the present time, for while some experiments have plainly pointed to a nitrogen assimilation of non

legumes, the results are somewhat indefinite, and it is difficult at present to determine the truth. Be this as it may, the fact still remains that it is to legumes chiefly that we must look for the restocking of our soils with nitrogen.

It has been a difficult matter to make any very valuable quantitative tests upon this power of bacteria and legumes to assimilate nitrogen from the air. Still, within the last year or two quite a number of extended experiments have been turned in this direction. Experiments have shown in the first place, that in regard to some of the legumes at least, a greater amount of nitrogen can be assimilated through the agency of the bacteria alone than can be assimilated by the same plants if they are fed with nitrogen foods. The same species of legumes are grown under two conditions, in both cases supplied with the organism which produces its root tubercles, and in the one experiment fed with nitrogen foods in the form of nitrates, and in the other not thus fed. The result shows a considerable difference to the advantage of the plants that are not fed with nitrates. The amount of nitrogen which can be assimilated and fixed in the soil by these legumes is really very great. In experiments with scarlet clover it has been found that a plant will assimilate from the air more than twelve times the amount of nitrogen in the seed. In one of the most recent experiments it has been found that by the use of beans a single crop assimilates and fixes in the soil 225 pounds of nitrogen per acre, equivalent to about 1400 pounds of nitrate of soda. These figures are very striking and suggestive, and they show plainly what a very great agent in the fixation of nitrogen the legume plants can become when properly associated with their appropriate species of bacteria. Peas, beans, cow peas, alfalfa, vetch and clover appear thus far to be the most valuable plants for this purpose, but other legumes serve the same purpose.

These experiments promise to be of the most incalulable value to the agriculturist and to the agricltural interests of the world in the future. They offer to our farmers a means of getting nitrogen without going to the expense of buying it, of enriching their soil by simply cultivating upon it plants which experiment has shown most appropriate to the soils in question. They emphasize the value of clover as a crop to precede wheat, and ex-

plain the great nutritive value of clover hay. Indeed, bacteriologists are now beginning to wonder if it has not been through the agency of these micro-organisms that the large nitrate beds of the world have been deposited. The nitrate beds of Chili are vast in extent, and it has been already suggested that these beds owe their deposition to the agency of some microbes, to bacteria associated with higher plants which have grown in these localities in past ages. If so, we see even more forcibly that at the very foundation the life of the world is dependent on the action of bacteria.

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On the Casting-off of the Tips of Branches of Certain Trees.—Part II.

BY AUG. F. FOERSTE.

(PLATES CXLVII. AND CXLVIII.)

In "Gray's Structural and Systematic Botany," page 44, may be found the following passage:

"When a terminal bud is formed* this is commonly the strongest or among the stronger. But in many cases it habitually or commonly *fails to appear.*† In the Elm the bud axillary to the last leaf of the season takes its place. In the common Lilac a pair of buds, which were in the axils of the uppermost of the opposite leaves, seem to replace the terminal bud, which *seldom*† develops."

On page 49 are the following words:

"In other cases, on the contrary, the branches grow on *indefinitely* until arrested by the cold of autumn; . . . the later-formed upper portion most commonly perishing from the apex downward for a certain length *in the winter.*† The Rose and Raspberry, and, among trees, the *Sumac* and *Honey-Locust*, are *good illustrations of this sort.*"†

In the September (1892) number of this journal the writer had occasion to show that the reason why the terminal bud *fails to*

* Incidentally the author is chiefly referring to the scaly buds of ligneous plants.

† Not italicized in the original.